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Structure-Activity Relationships of Species-Selectivity in Acute Chemical Toxicity between Fish and Rodents

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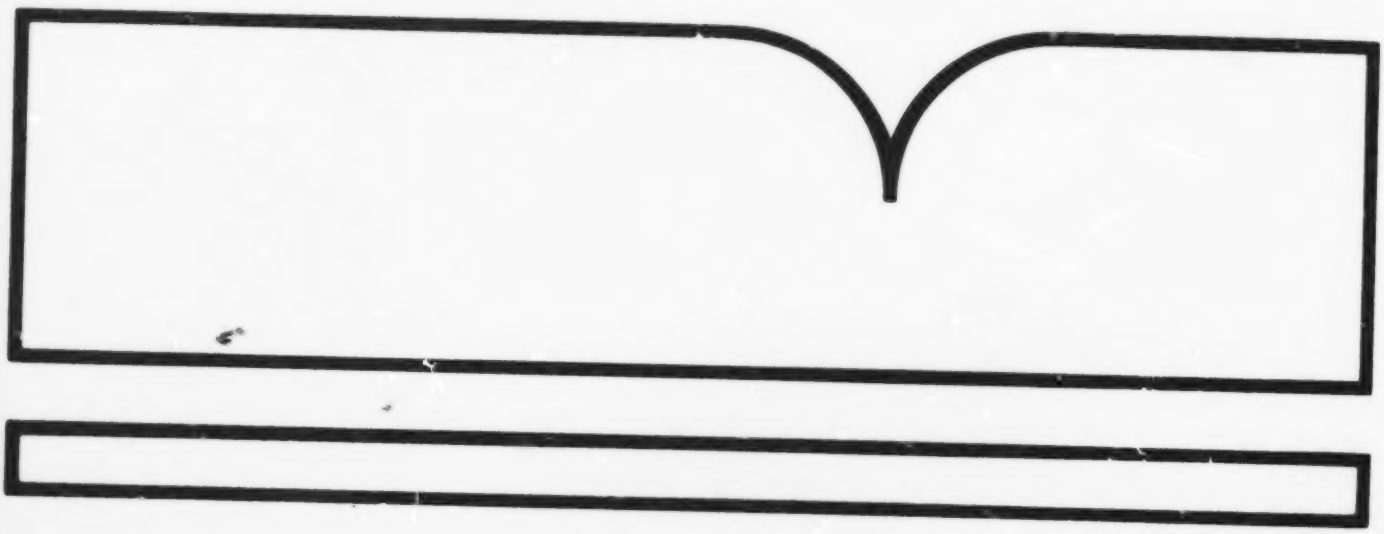

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16. ABSTRACT

Abstract – Although regression analyses between the acute oral or intraperitoneal LD50 values for both rats (*Rattus norvegicus*) and mice (*Mus musculus*) and the 96-h LC50 values for fathead minnows (*Pimephales promelas*) or rainbow trout (*Salmo gairdneri*) for a broad spectrum of environmental chemicals were statistically significant, the large variability in the regressions limits their practical utility for interspecies extrapolations. To gain a better understanding of this variability, analyses were performed to reveal the structural features characteristic of those chemicals that deviate substantially from the interspecies regressions. The criterion selected to indicate excessive variability was a greater-than-fourfold difference between the observed rodent LD50 and that estimated from the regression against the fish data. Discriminant function analyses showed aldehydes, esters and organophosphorus insecticides to be the primary outliers in the fish-to-rodent comparisons. Rodents were generally less sensitive to intoxication with carbonyl-containing compounds but more sensitive to organophosphate anticholinesterase poisoning than were fish. This species-selectivity for both classes of chemicals correlates with species-related differences in metabolic clearance and/or pharmacodynamic differences in target organ sensitivities. Since these same idiosyncracies in metabolic disposition and receptor sensitivity would be suspected to be operative for structurally related chemical compounds, interspecies extrapolations of toxicity information for both carbonyl compounds and for anticholinesterase agents should take into consideration these important factors, which confer certain degrees of species-selectivity for these chemical classes.

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Environmental Toxicology

STRUCTURE-ACTIVITY RELATIONSHIPS OF SPECIES-SELECTIVITY IN ACUTE CHEMICAL TOXICITY BETWEEN FISH AND RODENTS

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Abstract – Although regression analyses between the acute oral or intraperitoneal LD50 values for both rats (*Rattus norvegicus*) and mice (*Mus musculus*) and the 96-h LC50 values for fathead minnows (*Pimephales promelas*) or rainbow trout (*Salmo gairdneri*) for a broad spectrum of environmental chemicals were statistically significant, the large variability in the regressions limits their practical utility for interspecies extrapolations. To gain a better understanding of this variability, analyses were performed to reveal the structural features characteristic of those chemicals that deviate substantially from the interspecies regressions. The criterion selected to indicate excessive variability was a greater-than-fourfold difference between the observed rodent LD50 and that estimated from the regression against the fish data. Discriminant function analyses showed aldehydes, esters and organophosphorus insecticides to be the primary outliers in the fish-to-rodent comparisons. Rodents were generally less sensitive to intoxication with carbonyl-containing compounds but more sensitive to organophosphate anticholinesterase poisoning than were fish. This species-selectivity for both classes of chemicals correlates with species-related differences in metabolic clearance and/or pharmacodynamic differences in target organ sensitivities. Since these same idiosyncracies in metabolic disposition and receptor sensitivity would be suspected to be operative for structurally related chemical compounds, interspecies extrapolations of toxicity information for both carbonyl compounds and for anticholinesterase agents should take into consideration these important factors, which confer certain degrees of species-selectivity for these chemical classes.

Keywords – Interspecies comparative toxicity Interspecies extrapolation LC50 LD50
Structure-activity relationships

INTRODUCTION

The Toxic Substances Control Act delegates responsibility to the U.S. Environmental Protection Agency (EPA) for assessing the environmental impact and human health hazards of chemical compounds discharged or under consideration for discharge into the environment. This mandate requires the consideration of the multitude of animal species at potential risk of exposure. Time and economic constraints prohibit comprehensive toxicity testing of all species for each chemical. Accordingly, effective regulation depends on minimizing the number of required test organisms by identifying representative indigenous species for a defined biota. One way to accomplish this is to derive interspecies correlations of acute chemical toxicities, with the intent of identifying the most sensitive species to serve as sentinels for a given exposure medium [1–5]. Correlations of acute toxicities between unrelated species also provide a means for estimating acute health hazards in species for which limited information exists. Varying degrees of interspecies homology have been observed, with the more closely related taxa yielding the best correlations [4,6].

Interspecies correlations between aquatic organisms have been examined extensively and an exhaustive collection of acute toxicity values for several aquatic species has evolved [2–7]. An emerging interest in using aquatic data to assess risks to higher vertebrates has prompted correlation analyses of acute chemical toxicities between various aquatic test organisms and common laboratory mammals [8–11]. Despite initial reports of poor correlations for the acute toxicities of pesticides between fish and mammals [6,9], Kenaga [6]

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suggested that the correlations would be improved if they were restricted to structurally related chemical classes. Janardan and associates [11] provided additional evidence for structure-specific acute lethality correlations between rats and either fathead minnows or bluegills for selected priority pollutants and chlorinated hydrocarbon pesticides. However, the variability in the regressions limited the predictive capabilities to order-of-magnitude estimations.

The present investigation was designed to further assess the chemical determinants of species specificity in acute lethality between fish and common laboratory rodents for a wide array of environmental compounds. The specific objectives were to identify structural or physical-chemical characteristics of those compounds that failed to correlate with the regressions and to offer explanations for the interspecies variability. Identification of characteristics common to those compounds failing to conform to the regressions may provide insight into important determinants of species selectivity and mechanisms of toxicity. Clarification of these relationships also allows for more accurate estimates of acute mammalian toxicities for a broad spectrum of previously untested chemicals based on corresponding piscine data. Accordingly, the results provide additional information to assist in the effective evaluation of the health hazards posed by the expanding inventory of potentially toxic environmental chemicals.

METHODS

Fathead minnow (*Pimephales promelas*) 96-h flow-through median lethal concentration (LC50) values were provided by the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory–Duluth (ERL-D). All data were generated either in-house or under contract according to strict testing criteria established by the American Society for Testing and Materials (ASTM E729-80) as described by Phipps and Holcombe [5]. Data for a total of 341 chemicals were entered into the toxicity database at the time of analysis. This list of chemicals for which acute fathead minnow toxicity values were available served as the sole criterion for selecting chemicals for which the corresponding toxicity data for other species were extracted from the published literature. Acute toxicity values for rats (*Rattus norvegicus*), mice (*Mus musculus*) and rainbow trout (*Salmo gairdneri*) were obtained from searches of the literature as cited by the National Library of Medicine Toxline database or the EPA-National Institutes of Health Chemical Information System. Only those values for which the original manuscript was available for inspection were included in the analyses.

All LC50 and LD50 values were expressed as micromoles per liter aquarium water for fish and micromoles per kilogram body weight for rodents. When there were replicate citations for a single chemical, the geometric mean of the individual entries was used in the analyses. The records were then sorted to create subfiles according to species and route of administration. Comparisons were accomplished by merging the respective species-route subfiles.

Two additional databases were developed to complement the species files. The structure database consisted of a listing of descriptive functional groups for each chemical entry. The presence or absence of one or more of each of the following structural features was indicated: aromatic, alicyclic, or heterocyclic ring, unsaturated aliphatic bond, alcohol, ketone, aldehyde, carboxylic acid, ether, ester, amine, imine, nitrile, nitro, sulfo, halogen, or a phosphotriester group. One record was completed for each chemical entry. The third database consisted of a listing of physical-chemical constants for each chemical entry. Values for the molecular weight (mol. wt.), molar volume (mol. vol.), parachor, density in water (DW), boiling point (BP), and octanol-water partition coefficient (Po/w) were recorded as provided by the ERL-D.

The statistical methods used for assessing interspecies relationships of acute chemical toxicity consisted of least-squares linear regression of the logarithmically transformed acute toxicity values [12]. The criterion used to define excessive deviation from the interspecies comparisons was a greater-than-fourfold error between the observed acute mammalian LD50 and that predicted from the regression equation (95% confidence interval). A fourfold error represents about a 0.6 order-of-magnitude level of variability, which is less than that reported in the best examples cited by Kenaga [6] and less than the order-of-magnitude level of extrapolation reported by Janardan et al. [11]. Furthermore, in reviewing the citations contained in the database, it was not uncommon for the published LD50 for a single compound measured in one organism by different laboratories to differ by more than fourfold. For instance, the oral LD50 for chloroform in mice ranges from 36 to 2,180 mg/kg (60.5-fold) and the reported oral toxicity of acetonitrile in rats varies over a 42-fold range (160–6,690 mg/kg). In mice, the intraperitoneal (i.p.) LD50 for carbaryl (25 to 473 mg/kg) and

ethanol (557-9,200 mg/kg) differ by 19- and 16.5-fold, respectively, and the i.p. LD50 for parathion in rats has been reported to be between 0.6 and 17 mg/kg (41.8-fold range). Accordingly, although the selection of the fourfold range was somewhat arbitrary, this criterion was established solely to allow for distinguishing between those chemicals that conformed to the regressions and those that were outliers. It is not meant to imply that this degree of variability is in any way proposed as the acceptable level of variability for practical applications. The comparisons in the respective species files were then subdivided into three groups of chemicals: those conforming within the established error limits (on-line group), those that plotted above the upper (above-line) established limits of variability and those that plotted below the lower (below-line) established limits of variability.

Discriminant function analysis [13] was used to explore differences in the structural features and physical-chemical properties between the two non-conforming groups and the on-line group of chemicals. The primary objectives were to identify patterns of differences between the three groups and to select those variables (structural or physical-chemical) that could best discriminate between groups of chemicals. Because all of the physical-chemical values were not available for all chemicals, a series of discriminant analyses using the structural features and the physical-chemical variables was performed. This resulted in the inclusion of all chemicals for the analysis of structural features; however, chemicals were selectively excluded from analyses when there was a value missing for a physical-chemical variable.

Discriminant function analysis is a multivariate statistical technique [13] that identifies linear combinations of the original variables to maximize the separation between groups. The technique was well-suited for our purposes; however, the small sample size in some groups and the inability to satisfy the assumptions of normality for many variables (e.g., binary coding for the presence or absence of a specific structural feature) require that caution be used in interpreting the results. It is emphasized that the results were used to generate tentative hypotheses concerning the differential responses of fish and rodents to selected chemicals or chemical classes.

In several cases, the discriminant analyses revealed groups of outlier chemicals for which species-related differences in metabolic transformation could account for the large variability. Consequently, selected species-related comparisons of metabolic enzyme activity were performed in vitro for those enzymes suggested to be of significance and for which limited comparative data were available. Since the regressions revealed that both the carbonyl and organophosphate compounds exhibited a certain degree of species selectivity, the metabolic studies were limited to these two classes of agents. We recently reported a comparison of parathion metabolism in fish and rodents [14], and thus only the carbonyl-metabolizing enzymes were examined in this investigation.

Male Sprague-Dawley rats (175–200 g) and Swiss Webster mice (25–30 g) were purchased from Bio Lab Corp. (St. Paul, MN) and maintained in climate-controlled facilities on a 12-h light cycle with free access to food (Purina Chow) and water. Fathead minnows (2–4 g) were reared at the ERL-D. The rainbow trout (0.5–1.0 kg) were purchased from Cedar Bend Trout Farm (Scandia, MN) and maintained under closely regulated conditions at the ERL-D. The details of the fish rearing and handling conditions have been described by Phipps and Holcombe [5]. All animals were fasted for 12 to 48 h prior to killing by decapitation. The livers were excised into cold isotonic NaCl, weighed and homogenized (Polytron, Brinkmann Instruments) in four volumes (ml/g) of 50 mM sodium phosphate (pH 8.0). The homogenates were centrifuged at 4°C for 20 min at 9,000 *g* and the activities of the various enzymes in the resulting supernatant were determined. Each enzyme activity was measured at both 25°C and at the relevant physiological temperature (rat and mouse, 37°C; rainbow trout, 11°C).

Alcohol dehydrogenase activity was calculated from the ethanol-dependent rate of reduction of NAD [15]. The reaction mixture (2.4 ml) consisted of 100 mM sodium pyrophosphate (pH 8.8) containing 20 mM ethanol and 2.5 mM NAD. After allowing the mixtures to reach the appropriate temperature, the reaction was initiated by adding 0.1 ml of the 9,000 *g* supernatant (20 mg liver) and the rate of reduction of NAD monitored spectrophotometrically (E_{340nm} = 6.2 mM^{-1} cm^{-1}). The ethanol-dependent reduction of NAD was calculated by subtracting the rate observed in the absence of the substrate.

The activity of aldehyde dehydrogenase was measured spectrofluorometrically according to the method of Deitrich [16] and Weissbach et al. [17]. Indole-3-acetaldehyde bisulfite (10 mM) was preincubated in 0.95 ml 50 mM sodium-potassium phosphate (pH 7.4) containing 5 mM NAD. The reaction was initiated by adding 50 μl of the 9,000 *g* super-

natant (10 mg liver) and allowed to incubate at the appropriate temperature for 10 min. The incubation was stopped by adding 0.8 ml 0.125 N NaOH and 5 ml ethylene dichloride. The tubes were vigorously mixed for 3 min and the phases separated by centrifugation (2,000 *g* for 20 min). An aliquot (1.0 ml) of the upper aqueous phase was transferred to 50-ml screw-capped tubes containing 15 ml ethylene dichloride and 0.1 ml 6 N HCl. The tubes were sealed and shaken for 3 min prior to transferring 10 ml to clean tubes containing 3 ml 0.5 M sodium-potassium phosphate (pH 7.4). After additional mixing, the fluorescence (excitation, 285 nm; emission, 365 nm) of the aqueous layer was recorded. The recovery of indole acetic acid from the reaction mixture was measured daily and the calculated rates adjusted accordingly. A standard curve for the quantum efficiency of indole-3-acetic acid was included with each experiment. Preliminary experiments confirmed that the rate of the reaction was linear through 20 min of incubation.

Carboxylesterase activity was determined spectrophotometrically from the rate of hydrolysis of thiophenylacetate [18]. The equivalent of 0.2 mg liver (1 µl 9,000 *g* supernatant) was incubated in 2.4 ml 33 mM potassium phosphate (pH 7.4)-0.1 mM ethylenediaminetetraacetic acid (EDTA) containing 0.25 mM 5,5'-dithio-*bis*-2-nitrobenzoic acid (DTNB). After allowing the mixture to reach the appropriate temperature, the reaction was initiated by adding 1 mM thiophenylacetate and the absorbance at 412 nm was monitored for 10 min. The thiophenylacetate-dependent increase in absorbance was used to calculate esterase activity ($E = 13.6\ mM^{-1}\ cm^{-1}$).

Protein concentration was determined by the method of Lowry and associates [19]. Species-related differences in metabolic enzyme activities were analyzed by the randomized analysis of variance and the means compared by the unpaired Student's *t* test [12]. A probability of $p < 0.05$ was selected as the criterion for significance for all statistical analyses.

RESULTS

Rat or mouse, oral (p.o.) or intraperitoneal (i.p.) acute LD50s were located in the published literature for 138 (40%) of the 341 chemicals for which the 96-h LC50 was recorded for fathead minnows. The total number of chemicals for which data were obtained for each of the interspecies comparisons is indicated in Table 1. The range of toxic potencies for the chemicals included in the investigation spanned eight orders of magnitude for the fathead minnow LC50 (ethanol, 309 mmol/L; endrin, 1.7 nmol/L) and five orders of magnitude for the rainbow trout LC50 and for the LD50 for both rodents. The fathead minnow and rat p.o. comparison represented the largest subset, consisting of 100 individual chemical entries.

Regression analyses resulted in a significant

Table 1. Interspecies regression analyses

Variables[a]						
Independent	Dependent	No. of chemicals	Slope	Intercept	r^2 (%)	Standard error of estimate[b]
LC50 minnow	LD50 rat (p.o.)	100	0.320[c]	3.291	39	0.74
LC50 minnow	LD50 rat (i.p.)	37	0.343[c]	2.630	32	0.93
LC50 minnow	LD50 mouse (p.o.)	36	0.430[c]	2.724	67	0.62
LC50 minnow	LD50 mouse (i.p.)	56	0.277[c]	2.626	37	0.65
LC50 trout	LD50 rat (p.o.)	20	0.370[c]	3.163	29	0.91
LC50 trout	LD50 rat (i.p.)	11	0.419	2.602	29	1.11
LC50 trout	LD50 mouse (p.o.)	13	0.271	3.092	26	0.86
LC50 trout	LD50 mouse (i.p.)	12	0.451[c]	2.874	39	0.69
LC50 minnow	LC50 trout	19	0.868[c]	−0.436	81	0.69
LD50 rat (i.p.)	LD50 rat (p.o.)	28	0.970[c]	0.344	95	0.29
LD50 rat (i.p.)	LD50 mouse (i.p.)	23	1.076[c]	−0.197	89	0.37
LD50 rat (p.o.)	LD50 mouse (p.o.)	25	0.960[c]	0.251	91	0.36
LD50 mouse (i.p.)	LD50 mouse (p.o.)	26	1.061[c]	0.114	87	0.39

[a] Logarithmically transformed.
[b] Square root of error mean square.
[c] Statistically significant ($p < 0.05$).

relationship between the fathead minnow LC50 and the LD50 for both rodents, regardless of the route of administration. The rainbow trout LC50 correlated less well with the mammalian data, perhaps because of the limited number of chemicals for which corresponding toxicity data were available. Furthermore, the rainbow trout data were biased toward the more toxic and mechanistically specific pesticidal compounds. Regardless of the species, comparisons of the fish and mammal acute toxicity data generated regressions with slopes ranging from 0.27 to 0.45 and intercepts of approximately 1,000 μmol/kg. This contrasts with intraclass comparisons between species of fish or between species of rodents, which yielded slopes of approximately 1, suggesting proportionate rank-order sensitivities between closely related species. Intra-route comparisons of the acute LD50 for both rat and mouse yielded similar slopes of 0.97 and 1.06, respectively. Except for the rainbow trout versus rat i.p. and mouse p.o., all regressions were statistically significant ($p < 0.05$).

For the fathead minnow versus rat comparison, the acute oral LD50 for 68 of the 100 chemicals correlated with the fish data within the established range of variability (fourfold above or below). The oral toxicity for 19 compounds was less than that estimated from the fish values, whereas 13 of the chemicals were more toxic to rats than would have been projected. The results of the discriminant function analyses revealed three structural features that were consistently associated with chemicals that failed to conform within the limits of specified error for the regressions of acute toxicities between fish and rodents (Tables 2 and 3, Fig. 1).

Of the 19 chemicals that plotted above the upper limit of variability for the fathead minnow versus rat p.o. regression, 5 were aldehydes and 10 were carboxyl esters. Accordingly, 79% of those chemicals that were less acutely toxic to rats when administered orally than would be estimated from the fathead minnow data contained one or more carbonyl functional groups. Furthermore, all of the remaining aldehydes and esters plotted within the established prediction limits. Without exception, aldehyde and ester compounds invariably plotted either within or above the established limits of variability for all fish-mammal comparisons

Table 2. Discriminant function analysis of structure-specific patterns between conforming and nonconforming subgroups of chemical in the acute toxicity relationship between fathead minnows and rodents[a]

	Fathead minnow versus:											
	Rat oral			Rat intraperitoneal			Mouse oral			Mouse intraperitoneal		
Structural group	Above	On	Below	Above	On	Below	Above	On	Below	Above	On	Below
Total	19	68	13	9	21	7	3	28	5	10	37	9
Aromatic	2	27	5	5	11	3	1	12	3	4	20	4
Alicyclic	1	9	3	1	3	2	1	4	1	0	3	4[b]
Heterocyclic	0	7	1	0	1	2	0	3	1	0	3	3[b]
Alkene	5	3	1[b]	0	1	0	3	5	0[b]	4	3	0[b]
Alcohol	1	22	4	2	11	2	0	13	2	2	18	3
Ketone	0	11	2	0	1	1	0	2	1	0	3	1
Aldehyde	5	4	0[b]	0	0	0	0	0	0	0	0	0
Carboxylic	0	1	1	0	0	0	0	0	1[b]	0	1	0
Ether	0	10	1	0	4	0	1	5	0	0	5	1
Ester	10	8	0[b]	5	2	0[b]	3	6	0[b]	6	8	0[b]
Nitro	0	0	0	0	0	0	0	1	0	0	2	1
Amine	0	9	3	0	3	2	0	3	1	0	3	2
Imine	0	0	0	0	0	0	0	0	0	0	1	0
Nitrile	0	3	1	0	1	0	0	0	0	0	2	1
Sulfo	0	2	3[b]	0	2	3[b]	0	2	2	0	1	1
Halogen	2	19	5	2	9	1	1	12	1	2	9	2
Phospho ester	0	2	4[b]	0	1	4[b]	0	2	3[b]	0	2	2

[a] Values represent the number of chemicals containing one or more of the respective functional groups. The three subgroups of chemicals include those which plot within (On) the established limits of error and those which plot either above the upper (Above) or below the lower (Below) limits of prediction.
[b] Statistically significant ($p < 0.05$) structural discriminator of nonconformity in the interspecies regression among the three subgroups of chemicals.

Table 3. Discriminant function analysis of structure-specific patterns between conforming and nonconforming subgroups of chemicals in the acute toxicity relationship between rainbow trout and rodents[a]

	Rainbow trout versus:											
	Rat oral			Rat intraperitoneal			Mouse oral			Mouse intraperitoneal		
Structural group	Above	On	Below	Above	On	Below	Above	On	Below	Above	On	Below
Total	6	11	3	5	4	2	2	8	3	3	7	2
Aromatic	4	6	1	2	4	0[b]	1	6	1	1	5	1
Alicyclic	1	2	1	1	1	0	1	1	2	0	0	1
Heterocyclic	0	2	1	1	1	0	1	1	2	1	0	1
Alkene	3	1	0	0	0	0	1	1	0	1	1	0
Alcohol	0	1	0	1	1	0	0	2	0	0	2	0
Ketone	0	0	1[b]	0	1	0	0	0	1	0	0	1
Aldehyde	0	2	0	0	0	0	0	0	0	0	0	0
Carboxylic	0	0	0	0	0	0	0	0	0	0	0	0
Ether	2	1	0	1	0	0	0	1	1	0	0	0
Ester	3	2	0	1	1	0	2	2	0[b]	1	2	0
Nitro	0	1	0	0	1	0	0	1	0	0	1	0
Amine	0	0	0	0	0	0	0	0	0	0	0	0
Imine	0	0	0	0	0	0	0	0	0	0	0	0
Nitrile	1	0	0	0	0	0	0	0	0	0	0	1
Sulfo	0	2	3[b]	2	1	2	1	0	3[b]	1	0	1
Halogen	3	5	0	3	0	0	0	4	1	2	2	0
Phospho ester	0	3	3[b]	1	2	2	1	2	2	2	1	1

[a]Values represent the number of chemicals containing one or more of the respective functional groups. The three subgroups of chemicals include those which plot within (On) the established limits of error and those which plot either above the upper (Above) or below the lower (Below) limits of prediction.
[b]Statistically significant ($p < 0.05$) structural discriminator of nonconformity in the interspecies regression among the three subgroups of chemicals.

(Tables 2 and 3). Conversely, mammals tended to be more sensitive to the acute toxicity of organophosphate insecticides than would be projected from the fathead minnow data. A similar discrimination of species sensitivity did not apply to extrapolations from rainbow trout values because of the prevalence of highly toxic, specific-acting pesticides that biased the analyses.

Other functional groups that occasionally exhibited patterns of association for discriminating between the conforming and nonconforming groups of chemicals included alkenes, carboxylic acids, alicyclic and heterocyclic rings, ketones, and sulfur groups. However, the significance of these groups as determinants of species heterology is questionable because of the inconsistencies in the observations for the various interspecies comparisons.

The structure-specific regression of fathead minnow and rat p.o. toxicity is shown in Figure 1. The four non-carbonyl compounds that plotted above the upper limit of variability were *p-tert*-butylphenol, ethylbenzene, hexachloroethane and trichloroethylene. The nine non-organophosphates that were more toxic orally to rats than predicted from the fathead minnow data included sodium salicyclic acid, tripropylamine, allylcyanide, 1,3-diaminopropane, hexachlorophene, endrin, pentachlorophenol, 2-chloroethanol and chlordecone. The four organophosphates that deviated from the regression were parathion, azinphosmethyl, disulfoton and demeton.

Of the physical-chemical properties examined, no single parameter was consistently identified as a discriminator of noncompliance for the various interspecies comparisons (Tables 4 and 5). However, isolated cases of successful discrimination were observed for selected comparisons. In comparing the acute toxicity in fathead minnows with oral toxicity, those chemicals that were more toxic to rats than projected from the fish data exhibited a pattern of larger molecular weight, higher values of parachor and higher boiling points, whereas those chemicals for which rodent toxicity was overestimated by the fish data were of low molecular weight and had lower boiling points. In contrast, molecular volumes and density in water showed patterns of being potential discriminators

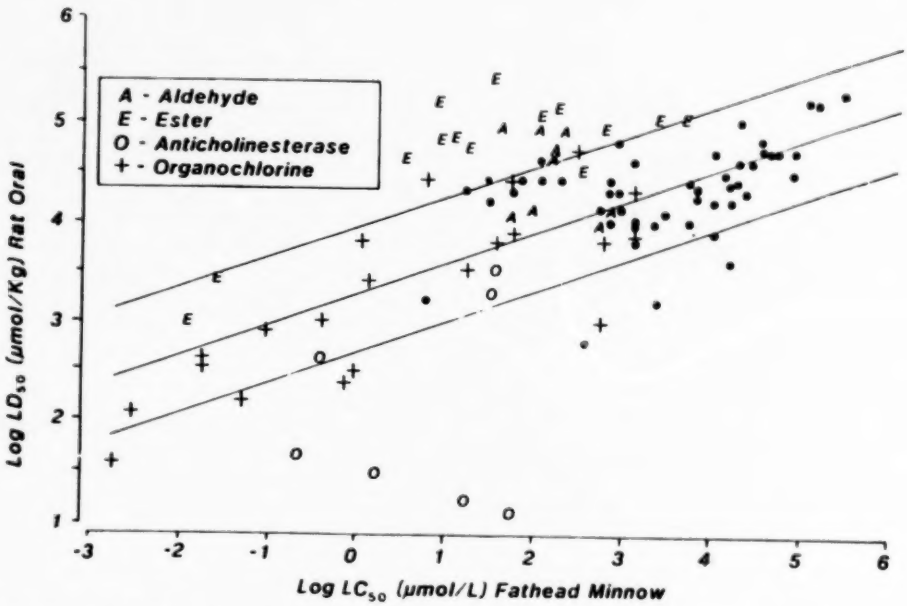

Fig. 1. Regression of the acute oral LD50 in rats against the 96-h LC50 for fathead minnows. Each point represents the coordinates for a single chemical compound. Solid circles represent those chemicals that lack a carbonyl, phosphoryl or organochlorine moiety. The geometric mean was calculated when duplicate values were reported for the LD50. The lines represent the calculated regression and the established fourfold prediction limits of error.

of nonconformity for the minnow-rat (i.p.) comparison. No other significant discriminating physical-chemical properties were observed for the other interspecies comparisons.

The greater rate of oxidative metabolism by rat and mouse liver homogenates correlates with the decreased sensitivity of rodents to aldehyde and carboxyl ester-induced lethality, as compared with fish (Table 6). Regardless of the incubation temperature, the activities of alcohol and aldehyde dehydrogenase and of carboxylesterase were significantly ($p < 0.05$) greater in rodents than in minnows when expressed either on the basis of tissue weight or per milligram protein. Hepatic alcohol dehydrogenase activities (per gram liver) measured at the respective physiological temperatures were 2- and 1.3-fold greater for rats and mice, respectively, than for fathead minnows. The activity of alcohol dehydrogenase in livers from rainbow trout was also greater (1.8-fold) than in minnows. Similarly, aldehyde dehydrogenase activity (per gram liver measured at physiological temperature) was 56-, 44-, and 3.3-fold greater in rats, mice, and trout, respectively, as compared with fathead minnows. Finally, carboxylesterase activities in liver from rats, mice, and trout were 24-, 12-, and 1.9-fold greater than in minnows. The species-related differences in hepatic metabolism could not be attributed solely to differences in reaction temperature. Although lowering the temperature of the reaction for the mammalian enzymes decreased the activities, mammalian enzyme activity measured at 25°C remained significantly greater than that in fathead minnows for all three enzymes examined. Similar to observations for mammalian enzymes, increasing the reaction temperature for trout resulted in enhanced activities for both hepatic alcohol-dehydrogenase and carboxylesterase.

DISCUSSION

Regressions of acute chemical toxicities between fish and mammals have been successful to varying degrees and have had implications for possible chemical class-specific relationships [6,9,11]. The regressions presented herein compare favorably with those reported by Janardan and associates [11] for fathead minnows and rats and provide species-specific determinants of variability in extrapolating from fish to rodents.

Unlike the intraclass or inter-route regressions,

Table 4. Discriminant function analysis between above-, on- and below-line groups of chemicals for physical-chemical properties in acute toxicity comparisons between fathead minnows and rodents[a]

Physical-chemical property	Fathead minnow versus:			
	Rat oral	Rat (i.p.)	Mouse oral	Mouse (i.p.)
Mol. wt.				
Above	136.38 ± 54.19(19)	241.34 ± 115.32(9)	220.55 ± 160.01(3)	169.09 ± 75.42(10)
On	155.20 ± 104.31(68)[b]	189.94 ± 108.70(21)	184.67 ± 103.77(28)	160.78 ± 85.48(37)
Below	248.25 ± 135.53(13)	212.86 ± 103.64(7)	221.51 ± 98.81(5)	204.40 ± 108.91(9)
Parachor				
Above	324.31 ± 114.51(19)	512.52 ± 190.91(9)	499.40 ± 307.27(3)	374.27 ± 184.14(10)
On	328.54 ± 168.65(67)[b]	363.70 ± 153.70(21)	353.11 ± 151.18(28)	320.05 ± 128.29(35)
Below	471.98 ± 190.70(12)	428.41 ± 207.76(7)	477.50 ± 209.34(4)	391.00 ± 174.15(9)
Mol. vol.				
Above	161.55 ± 58.06(19)	250.83 ± 93.23(9)	245.00 ± 145.59(3)	183.75 ± 91.79(10)
On	155.18 ± 77.03(65)	166.78 ± 65.50(20)[b]	157.90 ± 59.90(26)	144.45 ± 48.29(33)
Below	196.00 ± 95.23(8)	103.83 ± 49.79(3)	80.50(1)	160.50 ± 70.82(7)
DW (25°C)				
Above	7.94 ± 1.43(19)	6.56 ± 2.69(9)	6.51 ± 1.86(3)	7.89 ± 2.57(10)
On	8.50 ± 2.16(65)	7.97 ± 1.98(20)[b]	8.14 ± 1.72(26)	8.56 ± 1.91(33)
Below	7.62 ± 2.56(8)	10.46 ± 2.54(3)	11.41(1)	8.26 ± 2.27(7)
BP (K)				
Above	427.73 ± 58.71(19)	546.82 ± 127.02(9)	533.57 ± 186.31(3)	482.62 ± 102.67(10)
On	470.24 ± 113.95(64)[b]	513.72 ± 106.15(20)	499.01 ± 114.21(26)	490.00 ± 90.40(33)
Below	550.51 ± 170.39(8)	475.33 ± 217.09(3)	401.10(1)	546.21 ± 167.27(7)
Log Po/w				
Above	2.29 ± 1.17(19)	3.43 ± 1.94(9)	3.77 ± 2.98(3)	2.32 ± 1.51(10)
On	1.85 ± 1.89(67)	2.40 ± 1.98(20)	2.09 ± 1.96(27)	1.93 ± 1.69(37)
Below	2.61 ± 2.38(12)	2.04 ± 1.29(6)	2.25 ± 1.39(5)	1.93 ± 1.52(9)

[a]Values represent means ± SD. The number of chemicals in each group is indicated in parentheses. Individual subclasses consist of those chemicals plotting within the established range of acceptable error, or either above the upper or below the lower limits of prediction. Mol. wt., molecular weight; mol. vol., molar volume; DW, density in water; BP, boiling point; Po/w, octanol/water partition coefficient.
[b]Statistically significant ($p < 0.05$) discriminator for comparisons among the three subgroups of chemicals.

which yielded slopes of approximately 1, all fish-rodent regressions exhibited slopes of less than 1 (0.27 to 0.45). The slope of the regression line for the comparison of fathead minnow and rat p.o. data (0.32) agrees well with that reported by Janardan and colleagues [11]. Factors that may contribute to the slope's being less than unity include species-related differences in pharmacokinetics, such as metabolic transformation, and pharmacodynamic differences mediated at the level of interaction of the xenobiotic or its metabolite with the respective receptor molecule.

Although most of the interspecies regressions were statistically significant, the variability was sufficiently large to cast doubt on its practical use in extrapolating between species to predict acute toxicity. Discriminant analyses of the interspecies correlations revealed structural features characteristic of those chemical compounds that failed to conform within reasonable limits (fourfold above or below) of the regressions. An important observation was that many of these chemicals for which the rodent LD50 was underestimated from the corresponding fathead minnow data contained one or more carbonyl groups. Since metabolic oxidation of aldehydes and esters results in products having lower toxicities, the greater activity of the hepatic enzymes in rodents as compared with fish suggests a much more rapid metabolic clearance of carbonyl-containing compounds in the rodents. This greater capacity for metabolic detoxication may be a principal determinant of the relative insensitivity of rodents to the toxic effects of both aldehydes and esters. This hypothesis is supported by the work of Glickman and Lech [20], who suggested that the relatively greater sensitivity of rainbow trout to permethrin toxicity may be related to the slower rate of hydrolysis of the insecticide by carboxylesterases in the liver, kidney and plasma of trout as compared with rats.

Species-specific differences in susceptibility may also be related to the reactivity of the carbonyl

Table 5. Discriminant function analysis between above-, on- and below-line groups of chemicals for physical-chemical properties in acute toxicity comparisons between rainbow trout and rodents[a]

Physical-chemical property	Rainbow trout versus:			
	Rat oral	Rat (i.p.)	Mouse oral	Mouse (i.p.)
Mol. wt.				
Above	237.60 ± 144.23(6)	261.05 ± 151.32(5)	334.40 ± 5.73(2)	282.25 ± 101.33(3)
On	236.57 ± 125.90(11)	222.47 ± 98.19(4)	230.50 ± 107.50(8)	185.19 ± 65.81(7)
Below	288.68 ± 29.53(3)	274.36 ± 22.67(2)	327.52 ± 74.82(3)	191.69 ± 177.67(2)
Parachor				
Above	514.28 ± 312.30(6)	466.66 ± 250.00(5)	729.70 ± 86.69(2)	514.47 ± 231.53(3)
On	439.18 ± 202.16(11)	453.18 ± 167.42(4)	462.22 ± 208.60(8)	376.34 ± 141.08(7)
Below	591.83 ± 27.92(3)	583.95 ± 34.44(2)	593.03 ± 29.03(3)	384.80 ± 315.09(2)
Mol. vol.				
Above	249.08 ± 152.18(6)	197.75 ± 122.00(4)	385.00(1)	119.00(1)
On	178.50 ± 94.45(8)	157.50 ± 74.25(2)	203.58 ± 111.00(6)	167.42 ± 67.67(6)
Below	—(0)	—(0)	287.00(1)	77.00(1)
DW (25°C)				
Above	6.89 ± 2.54(6)	7.88 ± 3.06(4)	4.54(1)	9.07(1)
On	8.12 ± 2.70(8)	8.12 ± 2.31(2)	7.31 ± 2.13(6)	7.92 ± 1.95(6)
Below	—(0)	—(0)	5.40(1)	11.72(1)
BP (K)				
Above	536.50 ± 193.85(6)	531.28 ± 216.26(4)	701.80(1)	394.10(1)
On	521.90 ± 154.97(8)	513.90 ± 83.44(2)	532.98 ± 138.55(6)	493.90 ± 98.64(6)
Below	—(0)	—(0)	719.10(1)	579.90(1)
Log Po/w				
Above	4.46 ± 2.05(6)	3.13 ± 1.96(4)	4.26 ± 2.69(2)	3.57 ± 1.31(3)
On	2.75 ± 1.88(10)	2.48 ± 0.76(4)	3.83 ± 1.70(8)	2.86 ± 1.34(7)
Below	2.57 ± 0.25(2)	2.39(1)	2.57 ± 0.25(2)	0.78 ± 2.79(2)

[a]Values represent means ± SD. The number of chemicals in each group is indicated in parentheses. Individual subclasses consist of those chemicals plotting within the established range of acceptable error, or either above the upper or below the lower limits of prediction. Mol. wt., molecular weight; mol. vol., molar volume; DW, density in water; BP, boiling point; Po/w, octanol/water partition coefficient.

group, resulting in differences in target organ selectivity. The carbonyl oxygen atom is susceptible to protonation, especially under acidic conditions, to yield a reactive intermediate subject to nucleophilic addition. It is possible that when instilled into the acidic gastric contents, a large portion of the compound binds to nucleophiles resident on the surface of the gastric epithelium. Such a mechanism would result in less of the agent being available for absorption, thereby diminishing the systemic toxicity of the compound. Alternatively, in fish, the carbonyl group may undergo spontaneous activation at the surface of the gill, resulting in nucleophilic binding to the respiratory epithelium and, ultimately, asphyxiation.

Evidence suggestive of nucleophilic attack includes the indication of a structure-specific discrimination of noncompliance for both aldehydes and esters. Of the 18 esters in the fathead minnow-rat p.o. acute toxicity comparison, 13 were either mono- or diesters of aliphatic carbons and 5 contained one or more aromatic substituents. All 10 esters and 5 aldehydes that failed to fall within the limits of the regression were aliphatic esters. Conversely, all aldehydes and esters that contained one or more aromatic substituents conformed within the established limits of variability in the regression. Since electron-releasing alkyl substituents increase the reactivity of the transitional carbonyl oxygen (whereas a resonating aromatic structure stabilizes the carbonyl group), the more reactive aliphatic aldehydes and esters may be more liable to cause local caustic damage to the absorptive surface than a systemic response. Accordingly, the disproportionate distribution of aliphatic and aromatic carbonyl compounds suggests a possible nucleophilic-reactivity relationship resulting in altered target organ selectivity for the different species. Although this hypothesis is only speculative and we provide no substantiating evidence, the possible significance of this structure-activity relationship warrants additional investigation.

Of the 13 chemicals for which the acute oral toxicity in rats was underestimated by the regres-

Table 6. Metabolic oxidations catalyzed by liver in vitro[a]

	Alcohol-dehydrogenase		Aldehyde-dehydrogenase		Carboxylesterase	
	(nmol/min·g)	(nmol/min·mg)	(nmol/min·g)	(nmol/min·mg)	(μmol/min·g)	(nmol/min·mg)
Rat						
(37°C)	822.6 ± 26.8[b,c]	6.4 ± 0.3[c]	2,082.1 ± 227.1[b,c]	17.6 ± 1.4[b,c]	219.8 ± 12.4[b,c]	1,736.7 ± 66.9[b,c]
(25°C)	505.3 ± 36.6[b]	2.5 ± 0.4[b]	731.7 ± 223.3[b]	4.1 ± 1.4[b]	111.8 ± 12.7[b]	564.0 ± 128.9[b]
Mouse						
(37°C)	814.9 ± 120.8[b]	5.7 ± 0.9[c]	1,621.3 ± 63.7[b,c]	12.4 ± 1.3[b,c]	155.7 ± 13.2[b]	1,204.8 ± 188.4[b,c]
(25°C)	566.6 ± 79.1	2.7 ± 0.4[b]	267.4 ± 48.0[b]	1.3 ± 0.2[b]	139.2 ± 15.5[b]	675.9 ± 66.3[b]
Minnow						
(25°C)	392.8 ± 28.7	5.6 ± 0.4	37.0 ± 8.8	0.6 ± 0.2	9.0 ± 1.1	128.9 ± 9.7
Trout						
(11°C)	725.5 ± 111.8[b]	6.7 ± 0.8	122.5 ± 44.9	1.1 ± 0.4	17.3 ± 3.4[b]	160.6 ± 28.3
(25°C)	2,861.5 ± 656.0[b,c]	19.7 ± 3.1[b,c]	114.1 ± 41.3	0.9 ± 0.3	40.4 ± 2.6[b,c]	297.9 ± 20.0[b,c]

[a]Values represent means ± SE of 4 to 6 determinations and are expressed on both per gram liver and per milligram protein bases.
[b]Significantly different ($p < 0.05$) from the corresponding value for fathead minnows at 25°C.
[c]Significantly different ($p < 0.05$) from the corresponding value measured for the same species at 25°C.

sion with fathead minnow data, 4 were organophosphorus insecticides. This relatively greater sensitivity of rodents, as compared with fish, to poisoning by selected organophosphates, including parathion and guthion, has been reported by others [8,21,22]. In view of the biotransformation and selective mechanism of action of organophosphorothionates, species-related differences in susceptibility may reflect a combination of differences in metabolic activation or detoxication, differences in the sensitivity of tissue acetylcholinesterase to inhibition by the oxygen analogue, or species-specific differences in the rates of reactivation or aging of the inhibited enzyme. Both the rate of activation of phosphorothionate insecticides and the rate of detoxication of the active oxygen analogues are far more rapid in rats and mice than in several species of fish [8,14,21,23]. Since the effect of these opposing reactions is to diminish the significance of species-related differences in metabolic transformation as a determinant of differences in susceptibility to intoxication [14], subsequent investigations have focused on differences in acetylcholinesterase sensitivity.

It has been widely reported that the sensitivity of brain acetylcholinesterase to in vitro inhibition by various organophosphates is dramatically different between fish and rodents [8,24,25]. The concentration of paraoxon required to inhibit 50% of the enzyme in vitro is up to 200-fold greater for fish than for rats or mice and reflects both a greater affinity and a greater rate of phosphorylation of acetylcholinesterase in rodents [24,25]. This dramatic difference between species in the sensitivity of brain acetylcholinesterase to inhibition by organophosphates is consistent with the relative resiliency of fish to anticholinesterase intoxication. Alternatively, Weber and associates [26–28] suggested a lesser importance of acetylcholine in neuromuscular transmission in fish as compared with other vertebrates. They concluded that, in contrast to mammals, the toxicity of anticholinesterase insecticides in fish may not be mediated by the classical interference with nicotinic modulation of skeletal muscle function.

An interesting observation in this study is that two of the three anticholinesterase compounds that plotted within the limits of prediction for the fathead minnow-rat p.o. regression contained one or more carboxyl or carbamate esters (malathion and carbaryl, respectively). It can be postulated that, despite the greater sensitivity of acetylcholinesterase in rats to inhibition, a more rapid rate of hydrolysis of the esters to yield relatively inactive monoacids may account for the discrepancy between these compounds and other organophosphorus anticholinesterase insecticides. Accordingly, the same factors that contribute to the relative resiliency of rodents to ester-induced toxicity may explain why rodents are also less sensitive to carboxylester and carbamate insecticides than to other anticholinesterase compounds lacking a functional carbonyl group.

In conclusion, statistically significant relationships between the acute LC50 for fish and the acute LD50 for rodents for a broad spectrum of

environmentally relevant chemicals were observed. Deviations from the regressions could be explained on the basis of selected structural features of the individual compounds, and these structural features could be related to species-specific differences in either metabolic transformation and/or pharmacodynamic sensitivity. It appears, therefore, that for chemicals that are not metabolized to products having drastically altered toxic potencies and that do not elicit their toxic response by a specific mechanism, the acute mammalian LD50 may be estimated with reasonable accuracy from the corresponding fish data. However, judgments of interspecies comparisons are unpredictable at present for those compounds that are subject to metabolic activation or detoxication, or that elicit their toxic response by interacting with a high-affinity receptor. Consequently, consideration of species-related differences in both metabolic disposition and pharmacodynamic sensitivity is paramount in deriving accurate assessments of comparative toxicity.

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